R12

Lab 4 Isoascorbates

Investigations on the Toxic and Teratogenic Effects of GRAS Substances on the Developing Chick Embryo.[1]

Sodium Erythorbate

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[1]Report of investigations conducted under Contract No. 72-343 with the Food and Drug Administration, PHS, DHEW.

General Protocol:

Ten test substances were supplied by the Food and Drug Administration for testing in the chick embryo. Details on the nature and source of these substances is shown in Table i. All substances were stored at room temperature in the dark until they were used, except that the propyl gallate and phosphated mono- and di-glycerides were kept under refrigeration. Most of the substances were dissolved in a suitable solvent or suspended in a suitable liquid for injection into fertile eggs. In one instance the substance was injected directly without a solvent or carrier. Specific information about solvents, solubility of the substances and problems peculiar to individual substances will be given under specific protocol for each substance tested.

Fertile eggs used in these investigations were from a specific pathogen free flock of Dekalb 161 egg production type chickens fed a breeder ration free of antibiotics or other drugs. Eggs were stored at 55° F and a relative humidity of 80 percent for 0 to 5 days prior to use. Eggs were allowed to reach room temperature, placed on plastic flats and subjected to ultraviolet irradiation for 30 minutes. The top of each egg was cleansed by a cotton swab saturated with 70 percent ethanol, a small hole was drilled over the air cell through the shell and the test substance was injected with the aid of a 0.25 ml. tuberculin syringe fitted with a suitable needle. All equipment and glassware used to handle the test substances or their solutions or suspensions were sterilized by auto claving and every attempt was made to avoid microbiological contamination of the eggs. Following injection the hole in each egg was sealed by a drop of flexible collodion and the eggs were set in or returned to the incubators. Jamesway Model 252 Incubator-Hatchers were used and maintained at 100° F dry bulb temperature and 86° F wet bulb temperature during the first 18 days of incubation. Eggs were turned automatically each 4 hours. Eggs were candled periodically to remove dead embryos and all embryos were examined for stage of development and obvious defects. After 18 days of incubation viable embryos were transferred to hatching baskets and hatching temperature was reduced to 98.5° F dry bulb reading and humidity was increased to a 90° F wet bulb reading. Upon hatching (22nd day) chicks were examined for abnormalities and samples were cleared and alizarin stained to examine them for skeletal defects. Other embryos (50 for each substance studied) were sacrificed and samples of liver, muscle, bursa, brain, eye, spleen, heart, pancreas, lung and kidney were taken and fixed in formalin. Later tissues were embeded in paraffin, cut, stained and mounted for histopathological examination. Each sample was done in duplicate and hence a total of 10,000 tissues were examined for lesions.

Preliminary range finding experiments were conducted to find the doses of the test substances that could be used in constructing dose response curves for toxicity as measured by embryonic mortality. In two cases, the test substance was non-toxic in the largest dose that could be accommodated by injection. Specific dose response experiments using 100 or more eggs per dose and 5 or more doses of the test substance were conducted at a minimum of 3 time intervals to obtain the toxicity data reported. Solvent or sham injected controls and untreated control groups of eggs were used with each experiment. In some cases, extra trials were conducted to provide embryos for examination at critical doses of the test substances in order to further evaluate teratogenic response and obtain additional data on the nature of embryonic defects.

Data obtained from the experiments (except that from the range finding studies) was transferred to data sheets provided (FDH form 2572, 2572a and 2572b) and submitted to FDA for statistical analysis. Nine types of data summaries including 2 statistical treatments of the data were provided by FDA on the data submitted. The results presented and interpretations made are largely based on these data summaries.

Table i

FDA Project Test Substances

Test Substance and Identification	Compound No.
1. Lactose, Edible Formost Dairies, Inc. Appleton, Wisc.	000063423
2. Propyl Gallate Lot 337	000121799
3. Sodium Ascorbate, U.S.P. FCC Lot No. 965102 Hoffmann-LaRoche Inc., Nutley, N. J. FDA 3167 73(C)	000134032
4. Sodium Erythorbate F.C.C. Lot No. 834072 FDA 3167 73(C) Hoffmann-LaRoche, Nutley, N. J.	977052064
5. Oil Nutmeg NF, East Indian Fritzsche Dodge & Olcott, Inc. 71-28 New York, N. Y.	MX 8008455
6. Zinc Sulfate - Rayon Lot # 2132R1 Virginia Chemicals, Inc. Portsmouth, Va.	Anhyd. 007733020 Monohyd. 007446197
7. Stannous Chloride, AR $2H_2O$ Mallinckrodt Chemical Works St. Louis, Mo.	007772998
8. Talc USP #141, Whittaker, Clark and Daniels, Inc.	010101390
9. Carob Bean Gum FDA 71-14	PM 9000402
10. Phosphated Mono- and Di-Glycerides Lot No. 126 Witco Chemical Organics Division New York, N. Y. EMCOL D70-30C	977051323

General Discussion and Comparisons:

A comparison of the relative toxicity of the ten compounds tested is shown in Table ii. When toxicity is evaluated by the air cell route of injection at 96 hrs. of incubation, which was the most sensitive for most of the substances tested, it may be seen that the test substances can be divided into 3 categories of toxicity. Substances highly toxic are zinc sulfate, propyl gallate and carob bean gum. Moderate toxicity was encountered with sodium ascorbate, sodium erythorbate, oil of nutmeg and stannous chloride. Those substances of low toxicity were lactose, talc and phosphated mono- and di-glyceride.

Most of the substances tested produced general embryo toxic response as ascites and/or edema except for lactose and talc at the doses tested. Some specific structural defects were noted and seemed to be related to certain substances as shown in Table ii.

Table ii

Comparison of Ten Substances Tested
for Toxicity and Teratology

Substance Tested	LC_{50} via air cell at 96 hrs.	Specific Abnormalities Noted
Lactose	very large	none
Propyl Gallate	13 mgs./kg.	Ascites, edema, celosomia.
Sodium Ascorbate	100 mgs./kg.	Ascites, edema, celosomia, liver histopathology, head defects.
Sodium Erythorbate	84 mgs./kg.	Ascites, liver histopathology.
Oil of Nutmeg	240 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Zinc Sulfate	4 mgs./kg.	Ascites, edema, celosomia, dwarfism.
Stannous Chloride	120 mgs./kg.	Ascites, edema, celosomia.
Talc	>200 mgs./kg.	none
Carob Bean Gum	23 mgs./kg.	Anophthalmia, phocomelia, micromelia, torticollis, celosomia.
Phosphated Mono- and Di-Glycerides	>3000 mgs./kg.	Ascites, anophthalmia, brachygnathia.

IV. SODIUM ERYTHORBATE

Specific Protocol:

Sodium erythorbate is fairly soluble in water and hence solutions were made using this solvent. To prevent heat deterioration of the compound the solutions were filter sterilized. Maximum solubility concentrations of 150 mgs./ml. were used. Data for 5 doses of sodium erythorbate are presented at both 0 and 96 hrs. of incubation and via both air cell and yolk routes of administration. Supplementary trials were conducted with the injection of 100 µl. instead of 50 µl./egg to provide more data at higher dose levels. These data are not reported since they do not add to the interpretation of the results.

Results:

The data for sodium erythorbate is presented in Tables 13-16. When given at 0 hr. via the air cell a small but nonsignificant increase in percent mortality was noted at a dose of 7.5 mgs./egg. On the other hand, percent mortality was increased by a highly significant amount and the regression of dose on mortality was highly significant when the same amounts were given at 96 hrs. via the air cell. Yolk injection at 0 or 96 hrs. increased percent mortality at the two highest dose levels. As has been observed for sodium ascorbate the smallest dose of sodium erythorbate given at 0 hr. via the yolk appeared to decrease mortality but this effect is considered a chance event due to high solvent control mortality.

Percent abnormal chicks hatched was increased by 7.5 mgs. of sodium erythorbate per egg via the air cell at 0 hr. by a highly significant amount. This was also the case when 2.5, 5.0 or 7.5 mgs. were given via the air cell at 96 hrs. and when 1.0 or 7.5 mgs. were given via the yolk at 0 hr.

Percent H-S-V-L abnormalities was not significantly changed by any dose of sodium erythorbate or any condition of administration. Ascites and histopathology of the liver seems to be the major abnormalities increased in the chicks by treatment.

Disucssion:

Sodium erythorbate produced an embryo toxic effect that was closely related to the dose administered. The effect was evident in the high level of embryonic mortality and increase in percent abnormal chicks hatched, particularly when given at 96 hrs. via the air cell. The histopathological findings were not statistically significant because of their low incidence. The LC_{50} at 96 hrs. via the air cell was about 84 mgs./kg.

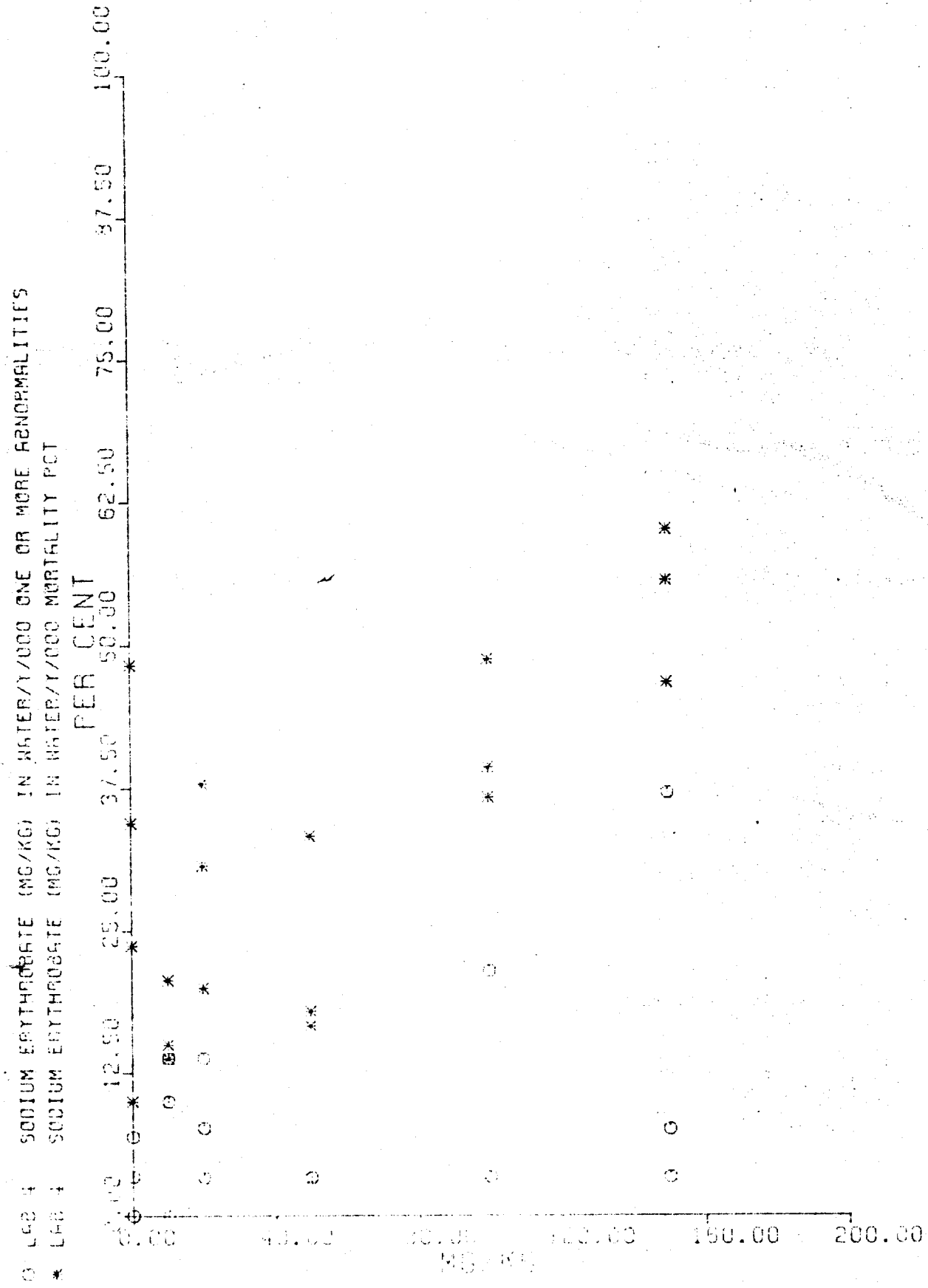
○ Lab 4 SODIUM ERYTHROBATE (MG/KG) IN RATS/6/000 ONE OR MORE ABNORMALITIES

✱ Lab 4 SODIUM ERYTHROBATE (MG/KG) IN RATS/6/000 MORTALITY PCT

PER CENT

12.50 25.00 37.50 50.00 62.50 75.00 87.50 100.00

* (A) : SODIUM EPITHOPROGIE (MG/KG) IN RATER/A.025 ONE OR MORE ABNORMALITIES
* (B) : SODIUM EPITHOPROGIE (MG/KG) IN RATER/A.025 MORTALITY PCT
PER CENT
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
150.00
200.00


O LAB 4 : SODIUM ERYTHROBATE (MG/KG) IN WATER/1/000 ONE OR MORE ABNORMALITIES
* LAB 4 : SODIUM ERYTHROBATE (MG/KG) IN WATER/1/000 MORTALITY POT
PER CENT
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
0.00
150.00
200.00

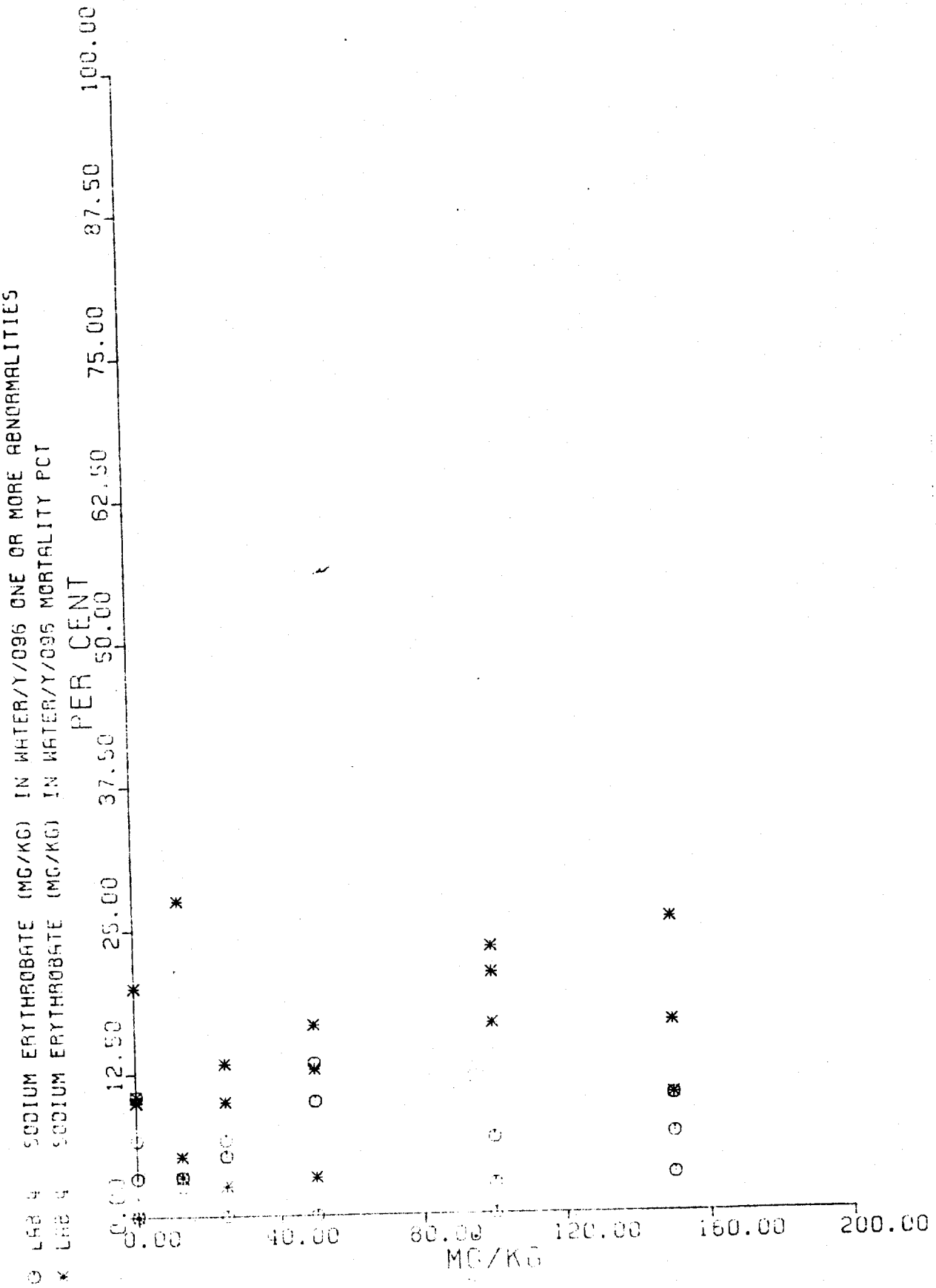

SODIUM ERYTHROBATE (MG/KG) IN WATER/Y/096 ONE OR MORE ABNORMALITIES
SODIUM ERYTHROBATE (MG/KG) IN WATER/Y/096 MORTALITY PCT
PER CENT
0.00
12.50
25.00
37.50
50.00
62.50
75.00
87.50
100.00
0.00
40.00
80.00
120.00
160.00
200.00
MG/KG

Table 13

DATA SUMMARY

Sodium Erythorbate in Water
via Air Cell at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	637	4.55	2.04	0.78
Solvent	None	159	6.91	3.77	1.88
10.0	0.5	100	4.00	1.00	0
20.0	1.0	100	6.00	1.00	0
50.0	2.5	100	5.00	6.00	0
100.0	5.0	100	5.00	2.00	0
150.0	7.5	98	14.28[1]	15.30[2]	0[3]

[1] NS

[2] Difference from control group response is highly significant

[3] NS

[4] F (Cal)< F (.05)

[5] Same as 4

Table 14

DATA SUMMARY

Sodium Erythorbate in Water
via Air Cell at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	637	4.55	2.04	0.78
Solvent	None	110	6.87	2.50	1.25
12.5	0.625	98	5.10	6.12	2.04
25.0	1.25	99	14.14	6.06	2.02
50.0	2.50	99	31.31[1]	18.18[2]	2.02
100.0	5.00	99	72.72[1]	14.14[2]	6.06[3]
150.0	7.50	99	65.65[1]	13.13[2]	3.03

[1] Difference from control is highly significant

[2] Difference from control group response is highly significant

[3] NS

[4] Regression of dose on mortality is highly significant

LC_{30} = 51 mgs./kg.
LC_{50} = 84 mgs./kg.
LC_{70} = 139 mgs./kg.
LC_{90} = 288 mgs./kg.

[5] F (Cal) < F (.05)

Table 15

DATA SUMMARY

Sodium Erythorbate in Water
via Yolk at 0 Hr.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	637	4.55	2.04	0.78
Solvent	None	126	28.57	2.38	0.79
10.0	0.5	98	16.32[1]	8.16	3.06
20.0	1.0	98	29.59	12.24[2]	1.02
50.0	2.5	99	22.22	3.03	0
100.0	5.0	97	42.26[1]	7.21	2.06
150.0	7.5	97	54.63[1a]	19.58[2]	4.12[3]

[1] Difference from control is significant

[1a] Difference from control is highly significant

[2] Difference from control group response is highly significant

[3] NS

[4] Regression of dose on mortality is significant

LC_{30} = 138 mgs./kg.
LC_{50} = 223 mgs./kg.
LC_{70} = 360 mgs./kg.
LC_{90} = 718 mgs./kg.

[5] $F\ (Cal) < F\ (.05)$

Table 16

DATA SUMMARY

Sodium Erythorbate in Water
via Yolk at 96 Hrs.

Dose of Compound Injected (mgs./kg.)	(mgs./egg)	Number of Eggs	Percent Mortality[4]	Percent Abnormal Chicks Hatched[5]	Percent H-S-V-L Abnormalities
Control	None	637	4.55	2.04	0.78
Solvent	None	158	12.98	6.33	2.00
12.5	0.625	96	11.45	8.33	1.04
25.0	1.25	98	8.16	4.08[2]	1.02
50.0	2.50	99	11.11	7.07	1.01
100.0	5.00	98	20.40[1]	7.14	3.06[3]
150.0	7.50	98	18.36	7.14	3.06

1 Difference from control is significant

2 NS

3 NS

4 Slope is negative

5 F (Cal) < F (.05)